

PATENT APPLICATION

of

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ANIMAL FOOD AND METHOD

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## ANIMAL FOOD AND METHOD

## CROSS REFERENCE TO RELATED APPLICATIONS

- 5                   This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/208,616, filed June 1, 2000.

## BACKGROUND OF THE INVENTION

- The present invention relates to compositions and methods for  
10   improving performance of breeding populations of swine, in particular by increasing reproductive performance. These improvements enhance commercial value of swine populations. More particularly, this invention is directed to administration to both sows and boars of a feed composition containing marine animal products from which are derived long chain omega-3 fatty acids, such as eicosapentaenoic acid,  
15   docosahexaneic acid, and docosapentaenoic acid, to increase the reproductive performance of breeding populations of swine.

- Omega-3 and omega-6 fatty acids and their metabolites regulate numerous activities *in vivo*, including inflammation, disease resistance, platelet function and vessel wall contractions. Moreover, supplementation of omega-3 fatty  
20   acids and/or gamma-linolenic acid present in the diet of animals and humans are reported to have favorable effects on growth, heart disease, inflammatory and autoimmune disorders, diabetes, renal disease, cancer, and immunity as well as learning, visual acuity and neurological function.

- On a cellular level long chain omega-3 fatty acids are readily  
25   incorporated into the phospholipid fraction of cell membranes where they influence membrane permeability/fluidity and transport. This represents a storage form of these fatty acids, where they remain until acted upon by phospholipase enzymes which release them for further conversion to eicosanoids.

- Linoleic and alpha-linolenic acids are C<sub>18</sub>-containing fatty acids that  
30   are parent compounds of the omega-6 and omega-3 families of fatty acids, respectively. Omega-3 and omega-6 fatty acids undergo unsaturation (i.e., adding double bonds) and sequential elongation from the carboxyl end (i.e., adding 2-carbon units) with the D6-desaturase enzyme being the rate limiting enzyme in metabolism of

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these long chain fatty acids. The same enzymes are used for these families, making the families antagonistic to one another. Such antagonism, resulting from requirements for the same enzymes, extends into the further metabolism of the C<sub>20</sub>-containing members of these families into metabolites called eicosanoids.

5                   The polyunsaturated fatty acids, including omega-3 and omega-6 fatty acids, differ from the other fatty acids in that they cannot be synthesized in the body from saturated or monounsaturated fatty acids, but must be obtained in the diet. The omega-6 fatty acid, linoleic acid, is found in high quantities in vegetable oils such as corn, cottonseed, soybean, safflower and sunflower oil. The omega-3 fatty acid,  
10   alpha-linolenic acid, is found in high quantities in flaxseed oil, linseed oil, perilla oil and canola oil. Other important compounds include arachidonic acid, found in animal fat; gamma-linolenic acid, found in evening primrose oil, borage oil, and blackcurrant oil; and eicosapentaenoic acid, docosahexaenoic acid, and docosapentaenoic acid derived from fish oils and marine algae. These long-chain fatty acids can be formed  
15   in the body by elongation and desaturation of the parent linoleic and alpha-linolenic acids if the parent compounds are supplied in the diet.

                  Various oils have been used as sources of omega-3 and omega-6 fatty acids in animal feed. The lactational responses of dairy cows fed unsaturated fat from extruded soybeans or sunflower seeds have been studied (Schingoethe, *et al.*, 1996);  
20   flaxseed oil has been used in animal feed to increase the number of live births in sows, to increase the number of live weaned pigs, and to allow for earlier breeding (U.S. Pat. No. 5,110,592); conjugated linoleic acid has been used in animal feed to increase fat firmness, shelf life, and meat quality (U.S. Pat. No. 6,060,087); linseed oil and corn oil have been used in animal feed as a source of omega-6 fatty acids to increase  
25   the number of live births and to increase the number of weaned pigs (Quackenbush, *et al.*, 1941); salmon oil has been used in pet food to reduce damage to skin and mucosa in animals, such as dogs and cats, where the animal is afflicted with cancer and is subjected to radiation therapy (U.S. Pat. No. 6,015,798); the effects of linseed oil, and omega-3 fatty acids in particular, on increased sperm fertility and female fertility,  
30   applicable to cattle, sheep, and rats, has been studied (Abayasekara, *et al.*, 1999); modified tall oil supplemented swine animal feed has been used to improve the carcass characteristics of swine and to increase daily weight gain (U.S. Pat. No.

6,020,377); the use of salmon oil to increase sperm fertility in roosters using a 1.5:1 ratio of omega-6 fatty acids to omega-3 fatty acids has been studied (Blesbois, *et al.*, 1997), and the effect of dietary fatty acids on lactic acid bacteria associated with the epithelial mucosa has been studied (Ringo, *et al.*, 1998).

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#### SUMMARY OF THE INVENTION

Although a number of favorable effects of omega-3 and omega-6 fatty acids have been reported in animals, there has been no previous suggestion that the administration of a composition of omega-3 fatty acids or esters thereof preferably  
10 derived from marine animal products can be used to increase the reproductive performance of swine, as is described and claimed in accordance with the present invention.

The present invention is based in part on the inventors' discovery that marine animal products, including fish oils such as salmon oil, containing long chain  
15 omega-3 fatty acids, administered to a female swine in a feed composition may cause several surprising and unexpected results. A feed composition including marine animal products may result in several benefits including an increase in the number of live births to the female swine in the first parity, an increase in the number of total births to a female swine, a decrease in the interval from weaning to estrus for female  
20 swine, increases in the uniformity of birth weight of offspring of female swine, decreases in pre-weaning death loss of the offspring of female swine, and an increase in the farrowing rate for female swine. The feed compositions of the present invention containing marine animal products also decrease the percentage of morphologic sperm abnormalities in male swine, which should increase the fertility of  
25 male swine.

In an embodiment of the present invention a method is provided for increasing the reproductive performance of a female swine. The method comprises the step of administering to the female swine a biologically effective amount of a feed composition comprising marine animal products containing omega-3 fatty acids or  
30 esters thereof that serve as a source of metabolites in the female swine to improve reproductive performance of the female swine. Methods and compositions of the present invention may serve to increase the reproductive performance of a female

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swine by causing any of the aforementioned benefits. A “biologically effective amount” is that amount that produces the desired effect. Examples of biologically effective amounts are provided herein, but those of skill in the art can readily adjust dosages depending on the type of swine, e.g. genotype or lines, the desired effect, the time period of administration, and the like, by using the methods disclosed herein.

The marine animal product may include a fish oil, in particular a fish oil from a North Atlantic cold water fish, such as salmon oil, or may be fish meal or an oil derived from fish meal, or a mixture thereof. The marine animal product serves as a source of omega-3 and omega-6 fatty acids. In a preferred embodiment of the invention the omega-6 fatty acids/esters to omega-3 fatty acids/esters ratio in the feed composition is from about 3:1 to about 20:1.

In another embodiment of the present invention, a method is provided for decreasing the percentage of morphological abnormalities in sperm, which should increase the fertility of a male swine. The method comprises the step of administering to the male swine a biologically effective amount of a feed composition that includes oils containing omega-3 fatty acids or esters thereof that serve as a source of metabolites in the male swine to increase fertility of the male swine. The oil specified in this method may be a marine animal product, for example, a fish oil such as salmon oil, or any other oil that provides a source of omega-3 and omega-6 fatty acids. The oil may also be added to the feed composition in the form of fish meal, an oil derived from fish meal, a plant oil, or an oil derived from ground seed, or a mixture thereof. In a preferred embodiment of the invention the omega-6 fatty acids/esters to omega-3 fatty acids/esters ratio in the feed composition is from about 3:1 to about 20:1.

In yet another embodiment of this invention, a method is provided for increasing the reproductive performance of a breeding population of swine by administering the feed composition of the present invention to both sows (females) and boars (males). The method includes the steps of administering to a female swine a biologically effective amount of a feed composition that includes marine animal products containing omega-3 fatty acids or esters thereof that serve as a source of metabolites in the female swine to improve reproductive performance of the female swine and administering to a male swine a biologically effective amount of a feed composition including oils containing omega-3 fatty acids or esters thereof that serve

as a source of metabolites in the male swine to decrease the percentage of morphological abnormalities in sperm, and decrease the number of rejected ejaculates which results in increased fertility of the male swine.

In another embodiment of the invention a swine feed composition is provided. The composition includes an animal feed blend and marine animal products wherein the marine animal products include a fish oil, such as salmon oil, a fish oil derived from fish meal, or fish meal products, or a mixture thereof, as a source of omega-6 and omega-3 fatty acids and their esters. Alternatively, the swine feed composition may include a plant oil, other than flaxseed oil, or a plant oil derived from ground seed. An example of a biologically effective feed composition is a composition containing about 0.025% to about 2% by weight of salmon oil, and the ratio of omega-6 fatty acids/esters to omega-3 fatty acids/esters in the feed composition is from about 3:1 to about 20:1. Another example of a biologically effective feed composition is a composition containing about 0.025% to about 1% by weight of salmon oil, and the ratio of omega-6 fatty acids/esters to omega-3 fatty acids/esters in the feed composition is from about 3:1 to about 20:1.

In an alternate embodiment of the present invention, a swine feed composition comprising an animal feed blend and marine animal products from which are derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, docosahexaneic acid, and docosapentaenoic acid or a mixture thereof is provided.

In another embodiment of the present invention, a method is provided for increasing the reproductive performance of a female swine. The method comprises the step of administering to the female swine a biologically effective amount of a feed composition comprising marine animal products from which are derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, docosahexaneic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the reproductive performance of the female swine. The method may serve to increase the reproductive performance of female swine by any of the benefits to the female swine described above. Examples of a "time sufficient" are disclosed herein and also may be readily determined by those of skill in the art using the methods disclosed herein.

In another embodiment of the present invention a method is provided for decreasing the percentage of morphologically abnormal sperm, and decreasing the percentage of rejected ejaculates which results in increased fertility of male swine.

The method comprises the step of administering to the male swine a biologically effective amount of a feed composition including an oil from which are derived omega-3 fatty acids. Suitable omega-3 fatty acids include eicosapentaenoic acid, docosahexaneic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the fertility of the male swine.

In yet another embodiment of the present invention, a method is provided for increasing the reproductive performance of a breeding population of swine. The method comprises the steps of administering to a female swine a biologically effective amount of a feed composition comprising marine animal products from which are derived omega-3 fatty acids selected from the group consisting of eicosapentaenoic acid, docosahexaneic acid, and docosapentaenoic acid or a combination thereof wherein the composition is administered for a time sufficient to increase the reproductive performance of the female swine and administering to a male swine a biologically effective amount of a feed composition including a biologically effective amount of an oil from which are derived omega-3 fatty acids. Suitable fatty acids include eicosapentaenoic acid, docosahexaneic acid, and docosapentaenoic acid or a mixture thereof wherein the composition is administered for a time sufficient to increase the fertility of the male swine.

To determine effects of the compositions of the present invention on swine reproductive performance, swine with similar genetic backgrounds are preferred. Optimal formulations may need some adjustments based on the genetic background of swine to be treated. Adjustments are preformed without undue experimentation by those of skill in the art.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Frequency distribution of litter size for control and salmon oil-treated female swine (data set 1).

Fig. 2 Frequency distribution of litter size for control and salmon oil-treated female swine (date set 2).

#### DETAILED DESCRIPTION OF THE INVENTION

- 5               Methods and compositions are provided for improving the performance of a swine breeding population by increasing the reproductive performance of female and male swine. The compositions of the present invention are lipid-containing compositions, and contain, in particular, marine animal products such as fish oil, fish meal, or an oil derived from fish meal, or combinations thereof.
- 10   The oils from marine animals, wherein "animals" include fish, serve as a source of omega-3 fatty acids/esters and omega-6 fatty acids/esters and their metabolites, such as eicosapentaenoic acid, docosahexaenoic acid, and docosapentaenoic acid or mixtures thereof. The compositions include omega-6 and omega-3 fatty acids or esters thereof present in the composition in a ratio of from about 3:1 to about 20:1.
- 15   Oils are understood to be lipids or fats including the glyceride esters of fatty acids along with associated phosphatides, sterols, alcohols, hydrocarbons, ketones, alkyl esters, salts, and related compounds.

- Fatty acids with no double bonds are termed saturated fatty acids, those with one double bond are termed monounsaturated fatty acids, and those with
- 20   multiple double bonds are termed polyunsaturated fatty acids. Overall digestibility appears to increase with the degree of unsaturation.

- A convenient shorthand system is used in this specification to denote the structure of fatty acids. This system uses a number denoting the number of carbons in the hydrocarbon chain, followed by a colon and a number indicating the
- 25   number of double bonds in the molecule, and then by a "w6" or a "w3" to denote "omega-6" or "omega-3," respectively (e.g., 22:5w6). The "w6" or a "w3" denotes the location of the first double bond from the methyl end of the fatty acid molecule. Trivial names in the w6 series of fatty acids include linoleic acid (18:2w6), gamma-linoleic acid (18:3w6), and arachidonic acid (20:4w6). The only fatty acid in the w3
- 30   series with a trivial name is alpha-linolenic acid (18:3w3). For the purposes of this application a fatty acid with the nomenclature 20:5w3 is eicosapentaenoic acid, with



the nomenclature 22:6w3 is docosahexaneoic acid, and with the nomenclature 22:5w3 is docosapentaenoic acid.

The methods of the present invention utilize a lipid-containing composition as a source of long chain omega-3 fatty acids, such as eicosapentaenoic acid, docosahexaneoic acid, docosapentaenoic acid, and esters thereof, to increase the reproductive performance of female and male swine. The reproductive performance of female animals may be increased by 1) increasing the number of live births to the female animal, 2) increasing the total births (i.e., live and dead offspring) to the female animal, 3) decreasing the interval from weaning to estrus (i.e., estrus is the period during which the female animal is capable of conceiving) for a female swine, 4) increasing the uniformity of birth weight of offspring of a female swine, 5) decreasing pre-weaning death loss of the offspring of a female swine, and 6) increasing the farrowing rate (i.e., the percentage of animals that give birth) for female swine.

The number of live births to a female animal may be increased by such mechanisms as enhancing follicular development, increasing the number of oocytes available for fertilization by sperm, increasing the viability of oocytes, increasing the susceptibility of oocytes to fertilization, increasing the viability of fertilized eggs, and reducing the mortality of embryos. These effects may result from changes in oocyte membrane integrity or lipid composition such that oocyte development, fertilization, or cell function (e.g., oocyte cell membrane transport, transmembrane signaling, or the regulation of intracellular signaling pathways in the oocyte) is altered to increase reproductive performance. An increase in long chain omega-3 fatty acids present in the tissues of the offspring at birth or obtained in the diet from the mother's milk may lead to an increase in the uniformity of birth weight of offspring of a female swine and/or a decrease in pre-weaning death loss of the offspring. A decrease in the interval from weaning to estrus for a female swine might result from changes in cellular metabolism due to the presence of long chain omega-3 fatty acids in the female animal's diet.

The reproductive performance of male animals may be increased by increasing the fertility of the spermatozoa of male animals. For example, the fertility of sperm may be increased by increasing the viability or motility of the sperm, by

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decreasing the percentage of abnormalities in the sperm (e.g., morphological abnormalities, abnormalities in staining intensity, and motility defects), or by increasing the numbers or the potency of sperm in male animals to which the lipid-containing composition is fed or otherwise administered. These effects may result from changes in sperm membrane integrity or lipid composition or in sperm cell function or development such that the performance of sperm to bind to and to fertilize an oocyte is increased.

A biologically effective amount of the lipid-containing composition must be administered to increase the reproductive performance of the animals. By “biologically effective amount” is meant an amount of the lipid-containing composition capable of increasing the reproductive performance of female or male animals by any mechanism, including those described herein.

The compositions of the present invention that contain marine animal products are preferably administered to swine orally in a feed composition, but any other effective method of administration known to those skilled in the art may be utilized. The feed composition may contain a marine animal product, such as a fish oil (e.g., salmon oil or another fish oil from a North Atlantic cold water fish), fish meal, or an oil derived from fish meal, or a mixture thereof, to provide a source of omega-3 fatty acids/esters and omega-6 fatty acids/esters in a mixture with an art-recognized animal feed blend.

The swine feed composition may be administered to the animals for any time period that is effective to increase the reproductive performance of swine. For example, the swine feed composition may be fed to the animals daily for the lifetime of a female or male animal. Alternatively, the swine feed composition may be administered to the female or male animal for a shorter time period. In a preferred embodiment of the invention, the swine feed is administered to a pregnant female animal for a period beginning about 1 to about 4 days prior to parturition (i.e., birth) and continuing through lactation (i.e., secretion of milk by the female animal) and through the next breeding until the female animal is impregnated a second time. In another preferred embodiment of the invention, the feed composition is administered to the female swine beginning about 30 days before a first mating of the female swine during an estrus and continuing through a second mating of the female swine during

the same estrus. In another preferred embodiment the feed composition is administered to the female swine beginning about 30 days before mating. In an alternate but equally preferred embodiment of the invention, the swine feed composition is administered to the female animal during lactation. The time periods for administration of the feed composition described above are nonlimiting examples and it should be appreciated that any time period determined to be effective to increase the reproductive performance of swine may be used.

Any animal feed blend known in the art may be used in accordance with the present invention such as rapeseed meal, cottonseed meal, soybean meal, and cornmeal, but soybean meal and cornmeal are particularly preferred. The animal feed blend is supplemented with a marine animal product as a source of omega-3 fatty acids/esters and omega-6 fatty acids/esters, but other ingredients may optionally be added to the animal feed blend. Optional ingredients of the animal feed blend include sugars and complex carbohydrates such as both water-soluble and water-insoluble monosaccharides, disaccharides and polysaccharides. Optional amino acid ingredients that may be added to the feed blend are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, tyrosine ethyl HCl, alanine, aspartic acid, sodium glutamate, glycine, proline, serine, cysteine ethyl HCl, and analogs, and salts thereof. Vitamins that may be optionally added are thiamine HCl, riboflavin, pyridoxine HCl, niacin, niacinamide, inositol, choline chloride, calcium pantothenate, biotin, folic acid, ascorbic acid, and vitamins A, B, K, D, E, and the like. Protein ingredients may also be added and include protein obtained from meat meal or fish meal, liquid or powdered egg, fish solubles, and the like. Any medicament ingredients known in the art may also be added to the animal feed blend such as antibiotics.

Antioxidants may be added to the feed composition to prevent oxidation of the fatty acids present in the marine animal products (e.g., fish oils) used to supplement the feed composition, such as the omega-3 long chain fatty acids, eicosapentaenoic acid, docosahexaenoic acid, and docosapentaenoic acid. Oxidation of fatty acids occurs over time and may be affected by such conditions as moisture and the presence of mineral catalysts and by such characteristics of fatty acids as the number of double bonds and positioning and configuration of bonds. Oxidation of

these omega-3 fatty acids can be prevented by the introduction of naturally-occurring antioxidants, such as beta-carotene, vitamin E, vitamin C, and tocopherol or of synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, tertiary-butylhydroquinone, propyl gallate or ethoxyquin to the feed composition.

- 5 Compounds which act synergistically with antioxidants can also be added such as ascorbic acid, citric acid, and phosphoric acid. The amount of antioxidants incorporated in this manner depends on requirements such as product formulation, shipping conditions (e.g., shipping under a nitrogen blanket), packaging methods, and desired shelf life.

- 10 The animal feed blend is supplemented with marine animal products such as fish oils, fish meal, fish oils derived from fish meal, other fish meal products, and the like, or a combination thereof. The fish oils may be obtained from any source, but a particularly preferred source is North Atlantic cold water fish. Fish oils obtained from North Atlantic cold water fish for use in accordance with the present invention
- 15 include salmon oil, menhaden oil, mackerel oil, herring oil, and the like, but fish oils from sources other than North Atlantic cold water fish may also be used in accordance with the present invention. Fish oils provide a source of both omega-3 and omega-6 fatty acids, but are a particularly good source of omega-3 polyunsaturated fatty acids. The omega-3 polyunsaturated long chain fatty acids eicosapentaenoic acid (20:5w3),
- 20 docosahexaneic acid (22:6w3), and docosapentaenoic acid (22:5w3) are typical of fish oil and together comprise about 25-38% by weight of the fish oil. Omega-6 polyunsaturated fatty acids present in fish oil include linoleic acid and arachidonic acid and are present at lesser concentrations of about 10% by weight. The oils or fatty acid ester components may be added in an unprocessed form or in pure form, may be
- 25 natural products or may be synthetic, and may be conjugated or unconjugated. The fatty acid esters added to the feed composition are preferably in the form of triglycerides, diglycerides, monoglycerides, phospholipids, lysopholipids, or are from natural sources and are chemically beneficiated for enhanced content of desired fatty acid esters.

- 30 The omega-6 fatty acids usable in the present invention are preferably unsaturated fatty acids having at least two carbon-carbon double bonds such as 2,4-decadienoic acid, linolenic acid, gamma-linolenic acid, 8, 10, 12-octadecatrienoic acid

and arachidonic acid. Gamma-linolenic acid is particularly preferred. The omega-6 fatty acids/esters to omega-3 fatty acids/esters ratio in the feed composition is from about 3:1 to about 20:1. It is to be understood that the ratio of omega-6 fatty acids/esters to omega-3 fatty acids/esters in the feed composition refers to the ratio in the final feed composition (i.e., the feed composition as a final mixture) containing the animal feed blend, the marine animal product (e.g., a fish oil), and any other oils or optionally added ingredients.

Omega-6 fatty acids/esters are present in fish oils at lower concentrations than omega-3 fatty acids/esters, and, thus, to achieve the ratios of omega-6 fatty acids/esters to omega-3 fatty acids esters of from about 3:1 to about 20:1 for use in the present invention, an additional source of omega-6 fatty acids/esters is generally used in the feed composition. Additional sources of omega-6 fatty acids/esters for use in the feed composition of the present invention include omega-6 fatty acids/esters derived from an art-recognized meal such as corn meal or soybean meal or from oils such as corn oil, cottonseed oil, soybean oil, safflower oil, sunflower oil, linseed oil, borage oil, blackcurrant oil, evening primrose oil, and the like.

The omega-3 fatty acids/esters and omega-6 fatty acids/esters may be administered to the female and male swine in the form of a marine animal product, such as fish meal, or preferably an oil, such as the fish oils and oils derived from fish meal described herein or mixtures thereof, wherein the oil is used as a supplement to an art-recognized animal feed blend. The oil predominantly contains esters of omega-3 and omega-6 fatty acids which are understood to be the glyceride ester precursors of the long chain omega-3 and omega-6 fatty acid metabolites, such as eicosapentaenoic acid, docosahexaenoic acid, and docosapentaenoic acid, which are believed to be the active form of the lipid molecule in accordance with the present invention. However, the oil may also contain small amounts of free omega-3 and omega-6 fatty acids such as C<sub>10</sub>- to C<sub>18</sub>-containing free fatty acids. The glyceride ester precursors are broken down intracellularly in the animal after adsorption through the gastrointestinal tract to form the free omega-3 and omega-6 fatty acids. The glyceride ester precursors present in the oils used in accordance with the present invention may also be glyceride ester precursors of C<sub>10</sub>- to C<sub>18</sub>-containing fatty acids that undergo unsaturation and

sequential elongation to form C<sub>20</sub>- to C<sub>22</sub>-containing long chain fatty acids, such as eicosapentaenoic acid (20:5w3), docosahexaenoic acid (22:6w3), and docosapentaenoic acid (22:5w3).

The feed composition of the present invention is supplemented with concentrations of a marine animal product, such as fish oil, fish meal, or an oil derived from fish meal, or combinations thereof, sufficient to provide amounts of omega-3 fatty acids/esters that are effective in increasing the reproductive performance of breeding populations of swine. For example, in a preferred embodiment of the invention the feed composition is supplemented with a fish oil containing omega-3 fatty acids/esters, such as salmon oil, in an amount of about 0.025% to about 1% by weight of the feed composition. In another preferred embodiment the feed composition is supplemented with a fish oil containing omega-3 fatty acids/esters, such as salmon oil, in an amount of about 0.025% to about 2%. In yet another preferred embodiment the feed composition is supplemented with a fish oil, such as salmon oil, in an amount of about 0.1% to about 0.5% by weight of the feed composition. In another preferred embodiment the feed composition is supplemented with a fish oil in an amount of about 0.2% to about 0.4% by weight of the feed composition. In each of these embodiments of the invention it is to be understood that the percentage of the fish oil by weight of the feed composition refers to the final feed composition (i.e., the feed composition as a final mixture) containing the animal feed blend, the marine animal product (i.e., the fish oil), and any other oils, and optionally added ingredients. In such embodiments of the invention, the fish oil may be derived from any type of fish or from fish meal.

In another embodiment of the invention, the feed composition is supplemented with a marine animal product such as fish meal. In one preferred embodiment the feed composition is supplemented with fish meal in an amount of about 1% to about 10% by weight of the feed composition. In another preferred embodiment, the feed composition is supplemented with fish meal in an amount of about 3% to about 4% by weight of the feed composition. It is to be understood that the percentage of fish meal by weight refers to the final feed composition containing the animal feed blend, the marine animal product, and any other oils, and optionally added ingredients.

The feed composition of the present invention can be administered to male swine or to female swine or to both male and female swine in a breeding population of swine to increase the reproductive performance of the animals. The feed composition administered to female swine is supplemented with marine animal products, such as a fish oil, fish meal, or a fish oil derived from fish meal, or a mixture thereof, in an amount sufficient to increase the reproductive performance of the female animals. The feed composition administered to male swine is preferably supplemented with a marine animal product, most preferably an oil, such as a fish oil, but fish meal, or an oil derived from fish meal, or combinations thereof, may also be used. For example, fish oils obtained from North Atlantic cold water fish including salmon oil, menhaden oil, mackerel oil, herring oil, and the like, may be used but the feed composition administered to the male swine may, alternatively, be supplemented with any other oil or source of omega-3 fatty acids/esters that is effective in increasing fertility of the male swine, including oils from any type of fish or plant oils.

Exemplary of other oils for use in supplementing the feed composition administered to the male swine as a source of omega-3 fatty acids/esters are linseed oil, perilla oil, canola oil, soybean oil, and the like. In another embodiment of the invention, the feed composition administered to the male swine is supplemented with an oil in the form of ground raw seed.

The marine animal products, such as fish oil (e.g., salmon oil), may be administered in an unencapsulated or an encapsulated form in a mixture with an animal feed blend. Encapsulation protects the omega-3 fatty acids/esters and omega-6 fatty acids/esters from breakdown and/or oxidation prior to digestion and absorption of the fatty acids/esters by the animal (i.e., encapsulation increases the stability of fatty acids) and provides a dry product for easier mixing with an animal feed blend. The omega-3 fatty acids/esters and omega-6 fatty acids/esters can be protected in this manner, for example, by coating the oil with a protein or any other substances known in the art to be effective encapsulating agents such as polymers, waxes, fats, and hydrogenated vegetable oils. For example, an oil may be encapsulated using an art-recognized technique such as a  $\text{Na}^{2+}$ -alginate encapsulation technique wherein the oil is coated with  $\text{Na}^{2+}$ -alginate followed by conversion to  $\text{Ca}^{2+}$ -alginate in the presence of  $\text{Ca}^{2+}$  ions for encapsulation. Alternatively, the oil may be encapsulated by an art-

recognized technique such as prilling (i.e., atomizing a molten liquid and cooling the droplets to form a bead). For example, the oil may be prilled in hydrogenated cottonseed flakes or hydrogenated soy bean oil to produce a dry oil. The oil may be used in an entirely unencapsulated form, an entirely encapsulated form, or mixtures of unencapsulated and encapsulated oil may be added to the feed composition. Oils other than fish oils may be similarly treated.

EXAMPLE 1: Salmon Oil Typical Analysis

|                                |                                |                 |                          |                 |
|--------------------------------|--------------------------------|-----------------|--------------------------|-----------------|
| 10                             | Free Fatty Acids<br>(as Oleic) | 3.0%<br>Maximum | Moisture &<br>Impurities | 1.0%<br>Maximum |
| Typical Fatty Acid Composition |                                |                 |                          |                 |
| 15                             | C10                            | --              | C18:4 (w3)               | 1.63            |
|                                | C12                            | < 0.10          | C20                      | 0.16            |
|                                | C13                            | < 0.10          | C20:1                    | 5.25            |
|                                | C14                            | 5.6             | C20:2 (w6)               | 0.28            |
|                                | C14:1                          | 0.13            | C20:3 (w6)               | 0.28            |
|                                | C15                            | 0.37            | C20:4 (w3/w6)            | 2.32            |
|                                | C15:1                          | < 0.10          | C20:5 (w3)               | 10.50           |
|                                | C16                            | 13.2            | C21:5 (w3)               | 0.69            |
|                                | C16:1                          | 9.0             | C22                      | < 0.1           |
|                                | C16:2                          | 1.15            | C22:1                    | 5.18            |
| 20                             | C16:3                          | 0.80            | C22:4 (w6)               | 0.20            |
|                                | C16:4                          | 0.67            | C22:5 (w3)               | 4.94            |
|                                | C17                            | 0.33            | C22:6 (w3)               | 11.0            |
|                                | C18                            | 2.81            | C24                      | < 0.1           |
|                                | C18:1                          | 17.3            | C24:1                    | 0.49            |
| 25                             | C18:2(w6)                      | 3.9             |                          |                 |
|                                | C18:3 (w3)                     | 1.8             |                          |                 |
| 30                             | Total Omega 3 Fatty Acids      | 31.76           |                          |                 |
|                                | Total Omega 6 Fatty Acids      | 5.66            |                          |                 |
|                                | Omega 3:Omega 6 ratio          | 5.61            |                          |                 |



EXAMPLE 2: Effect of Feed Composition Containing Fish Oil  
on Reproductive Performance of Female Swine

DATA SET 1:

5

LACTATION DIET

Control and treated sows were fed the diets shown below during lactation. During breeding sows were fed from the time of weaning to estrus.

10

| INGREDIENT                 | CONTROL | TREATED |
|----------------------------|---------|---------|
| Corn                       | 1139    | 1139    |
| 44 SBM*<br>(Soy bean meal) | 620     | 620     |
| Salmon Oil                 | 0       | 5       |
| Soy Hulls                  | 50      | 50      |
| Lact. Base 120             | 120     | 120     |
| Fat                        | 50      | 45      |
| Premix with<br>Antibiotics | 21      | 21      |

15

20

2000 lbs. 2000 lbs.

During estrus, sows were fed the following diet either to the first or second mating, if a second mating was necessary.

-17-

BREEDING DIET

| INGREDIENTS                | TREATED |
|----------------------------|---------|
| Corn                       | 1369    |
| 44 SBM*                    | 450     |
| Salmon Oil                 | 5       |
| Soy Hulls                  | 50      |
| Bache X<br>(breeding base) | 92      |
| Fat                        | 30      |
| Premix with<br>Antibiotics | 4       |

2000 lbs.

\*44% crude protein

## RESULTS

| TREATMENT | # OF SOWS | TOTAL<br>BORN | BORN<br>ALIVE | WEAN TO ESTRUS<br>(DAYS) |
|-----------|-----------|---------------|---------------|--------------------------|
| CONTROL   | 196       | 10.85         | 9.91          | 5.69                     |
| TREATED   | 181       | 11.27         | 10.42         | 5.69                     |

DATA SET 2:LACTATION DIET

| INGREDIENT              | CONTROL | TREATED |
|-------------------------|---------|---------|
| Corn                    | 1292.5  | 1287.5  |
| 44* SBM                 | 565     | 565     |
| Fat                     | 20      | 20      |
| Salmon oil              | -       | 5       |
| Lact. Base 120          | 120     | 120     |
| Premix with Antibiotics | 2.5     | 2.5     |

2000 lbs.      2000 lbs

\*44% crude protein

15 Sows were fed during lactation.

RESULTS

| TREATMENT | # OF SOWS | TOTAL BORN | BORN ALIVE | WEAN TO ESTRUS (DAYS) |
|-----------|-----------|------------|------------|-----------------------|
| CONTROL   | 126       | 11.50      | 10.39      | 5.98                  |
| TREATED   | 155       | 12.01      | 10.31      | 5.66                  |

EXAMPLE 3: Effect of Feed Composition Containing  
Fish Oil on Boars' Reproductive System

In preliminary studies, boar ration #1 was used. As a preliminary  
5 result, there was a 25-30% increase in the number of doses of semen produced by a  
treated boar. Most of this response was due to less of the boar's ejaculates being  
rejected from further processing, thus increasing the number of doses of semen  
produced. This had a significant increase in financial gains from this stud.

Another large test on the effects of the feed composition of the present  
10 invention on the reproductive system of boars was performed with approximately 220  
boars. The boars were first fed the control ration (ration #3) and ejaculates were  
examined for each boar. The boars were then fed boar ration #2 containing salmon oil  
and ejaculates were examined for each boar. The boars were fed the same diet during  
the "treatment" period as during the control period except for the addition of salmon  
15 oil at 5 lbs./ton of final diet. The effect of feeding boar ration #2 was that the number  
of rejected ejaculates was 50% less when the boars were fed boar ration #2 than when  
the boars were fed the control diet (ration #3). There were 86 rejected ejaculates  
when the boars were fed the control ration and 41 rejected ejaculates when the boars  
were fed ration #2. This is a significant savings and improved efficiency of producing  
20 doses of semen.

| FEED      | NO. OF BOARS | # OF REJECTED EJACULATES |
|-----------|--------------|--------------------------|
| Control   | 220          | 86                       |
| Treatment | 220          | 41                       |

25

30

35

TREATMENT RATIONSBOAR RATION #1

|    |              |                   |
|----|--------------|-------------------|
| 5  | Corn         | 1371.5            |
|    | Soybean Meal | 300.0             |
|    | Fish Meal    | 60.0              |
|    | Boar Base    | 268.5             |
| 10 |              | <hr/> 2000.0 lbs. |

BOAR RATION #2

|    |              |                   |
|----|--------------|-------------------|
| 15 | Corn         | 1326.5            |
|    | Soybean Meal | 400.0             |
|    | Salmon Oil   | 5.0               |
|    | Boar Base    | 268.5             |
| 20 |              | <hr/> 2000.0 lbs. |

CONTROL RATIONS

|    |              |                 |
|----|--------------|-----------------|
|    | Corn         | 1331.5          |
|    | Soybean Meal | 400             |
| 25 | Salmon Oil   | 0               |
|    | Boar Base    | 268.5           |
|    |              | <hr/> 2000 lbs. |

30                      Boars were fed individually once daily, and were kept separate from the females.

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Example 4: Effect of Feed Composition Containing Fish Oil on the Reproductive Performance of Female Swine

DATA SET 1:

5

Female swine were randomly assigned within parity to a control or a salmon oil containing diet upon entry to the farrowing room at approximately 110 days of gestation. Control sows received the lactation ration described below and treated sows received the same ration with 5 pounds of salmon oil replacing 5 pounds of fat.

10

LACTATION RATION

15

| Ingredient     | Control | Treatment |
|----------------|---------|-----------|
| Corn           | 1229.0  | 1229.0    |
| SBM, 48%       | 566.5   | 566.5     |
| Lact. Base 120 | 120.0   | 120.0     |
| Salmon Oil     | 0.0     | 5.0       |
| Fat            | 20.0    | 15.0      |
| Laxative Pak   | 10.0    | 10.0      |
| A-90           | 2.5     | 2.5       |
| Nutrisound     | 1.0     | 1.0       |
| Mold Inhibitor | 1.0     | 1.0       |
| Soy Hulls      | 50.0    | 50.0      |
| Total          | 2000.0  | 2000.0    |

20

25

Female swine in the treatment group continued on the salmon oil-containing diet following weaning during the rebreeding period, with 5 pounds of salmon oil included in the breeding ration (described below).

30

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BREEDING RATION

|    | Ingredient     | Control | Treatment |
|----|----------------|---------|-----------|
|    | Corn           | 1423.0  | 1423.0    |
| 5  | SBM, 48%       | 359.5   | 359.5     |
|    | Sow 93         | 93.0    | 93.0      |
|    | Salmon Oil     | 0.0     | 5.0       |
|    | Fat            | 20.0    | 15.0      |
|    | A-90           | 2.5     | 2.5       |
| 10 | Nutrisound     | 1.0     | 1.0       |
|    | Mold Inhibitor | 1.0     | 1.0       |
|    | Soy Hulls      | 100.0   | 100.0     |
|    | Total          | 2000.0  | 2000.0    |

15                   The salmon oil was stored in a 55-gallon metal drum. Each time feed was prepared, salmon oil was removed from the drum with a manual pump, weighed and placed in the mixer. Feed was immediately delivered to a designated storage tank adjacent to the farrowing rooms. Two feed tanks with associated auger system delivered feed to each farrowing room. Fresh feed was prepared at approximately one

20 week intervals, and feeding of the animals with a portion of the freshly prepared feed was initiated immediately. A similar procedure was followed for the breeding diet. Female swine were fed according to standard procedures, which consisted of female swine receiving 4 to 5 pounds of feed per day prior to farrowing and then increasing the amount of feed by approximately 2 pound per day post farrowing until the female

25 swine reached ad libitum intake. Female swine received approximately 6 pounds of feed per day during breeding.

                    Within 48 hours following farrowing, piglets were cross-fostered to equalize the number of piglets nursing on each sow. Piglets were fostered within and across treatments and were weaned at approximately 14 days after birth and the

30 female swine were then moved to breeding stalls for estrus detection and mating. All female swine were housed in gestation stalls and fed a common gestation ration until subsequent entry into the farrowing room.

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A total of 42 control and 45 treated sows were allotted to the trial. The total number of piglets born at the second farrowing was greater ( $p < 0.05$ ) for salmon oil treated (13.2) than control sows (11.7) as shown in the table below.

| 5  | Effect of salmon oil on sow reproductive performance |                   |                    |
|----|--|-------------------|--------------------|
|    |  | Control           | Treated            |
|    | Number of Sows                                       | 42                | 45                 |
|    | Wean to Estrus Interval                              | 5.6               | 5.1                |
|    | Number Farrowed                                      | 37                | 40                 |
| 10 | Total Pigs Born                                      | 11.7 <sup>a</sup> | 13.2 <sup>b</sup>  |
|    | Pigs Born Live                                       | 10.8 <sup>a</sup> | 12.35 <sup>b</sup> |

<sup>ab</sup> Means within a row without common superscripts differ significantly ( $P < 0.05$ ).

The frequency distribution for litter size is shown Figure 1. This frequency distribution shows a shift, with fewer litters of less than twelve piglets for treated female swine compared to controls and greater numbers of larger litters for treated female swine.

#### DATA SET 2:

The same procedures as described above were used except that a total of 109 control and 107 sows were allotted to the trial and the lactation and breeding rations described below were used.

| 25 | LACTATION RATION |         |           |
|----|------------------|---------|-----------|
|    | Ingredient       | Control | Treatment |
|    | Corn             | 1229.0  | 1229.0    |
|    | SBM, 48%         | 566.5   | 566.5     |
|    | Lact. Base 120   | 120.0   | 120.0     |
|    | Salmon Oil       | 0.0     | 10.0      |
| 30 | Fat              | 20.0    | 10.0      |
|    | Laxative Pak     | 10.0    | 10.0      |
|    | A-90             | 2.5     | 2.5       |
|    | Nutrisound       | 1.0     | 1.0       |
|    | Mold Inhibitor   | 1.0     | 1.0       |
| 35 | Soy Hulls        | 50.0    | 50.0      |
|    | Total            | 2000.0  | 2000.0    |



| BREEDING RATION |         |           |
|-----------------|---------|-----------|
| Ingredient      | Control | Treatment |
| Corn            | 1423.0  | 1423.0    |
| SBM, 48%        | 359.5   | 359.5     |
| Sow 93          | 93.0    | 93.0      |
| Salmon Oil      | 0.0     | 10.0      |
| Fat             | 20.0    | 10.0      |
| A-90            | 2.5     | 2.5       |
| Nutrisound      | 1.0     | 1.0       |
| Mold Inhibitor  | 1.0     | 1.0       |
| Soy Hulls       | 100.0   | 100.0     |
| Total           | 2000.0  | 2000.0    |

The total number of piglets born at the second farrowing was greater ( $p < 0.05$ ) for salmon oil treated (11.76) than control sows (10.67) as shown below. The frequency distribution for litter size is shown in Figure 2. This frequency distribution shows a shift, with fewer litters of less than eleven pigs for treated female swine compared to controls and a greater number of larger litters for treated female swine.

| Effect of salmon oil on sow reproductive performance |                    |                    |
|--|--------------------|--------------------|
|  | Control            | Treated            |
| Number of Sows                                       | 109                | 107                |
| Wean to Estrus Interval                              | 6.28               | 6.25               |
| Number Farrowed                                      | 85                 | 88                 |
| Total Pigs Born                                      | 10.67 <sup>a</sup> | 11.76 <sup>b</sup> |
| Pigs Born Live                                       | 9.81 <sup>a</sup>  | 10.76 <sup>b</sup> |

<sup>ab</sup> Means within a row without common superscripts differ significantly ( $P < 0.05$ )

#### Example 5: Effect of Prilling on Stability of Omega Fatty Acids in the Feed Composition

Salmon Oil was prilled (i.e., converted from a molten liquid and atomized into droplets to form a prill, or a bead) to produce a 35% w/w salmon oil prilled concentrate. The prilled concentrate was mixed at 10.75% w/w with animal feed. Samples (about 0.5 kg) of prilled concentrate (no feed), control feed (lactation

diet composition as described in Example 4) without salmon oil, control feed plus prilled concentrate, control feed plus liquid salmon oil (not prilled), and a premix (a vitamin and mineral supplement) with liquid salmon oil were collected and were analyzed for omega fatty acid content over an 8-week period. Prilled concentrate, control feed, and control feed plus prilled concentrate samples were collected on the day of manufacture and were analyzed within 7 days of manufacture (designated as “Wk 0”). Products were sampled at 4-week intervals for fatty acid analysis (designated “Wk 4” and “Wk 8”). Samples of control feed plus liquid salmon oil and premix plus liquid salmon oil were collected and analyzed approximately four weeks after manufacture (designated “Wk 4”). Samples of the control feed plus liquid salmon oil and premix plus liquid salmon oil were collected and analyzed again four weeks later (designated “Wk 8”). The samples were analyzed for omega fatty acid content by using art-recognized techniques for lipid extraction (“Mojonnier Method,” A.O.A.C. 954.02, 15<sup>th</sup> Edition, 1990) and fatty acid analysis (determined by gas chromatography-A.O.C.S. *Ce1e-91* and *Ce1d-91* for omega fatty acids).

The results are shown in the table below. The data for the salmon oil-containing feed and salmon oil-containing premix samples are expressed as % by weight (i.e., g/100g). The results demonstrate that the omega fatty acids in prilled salmon oil from a mixture with animal feed are stable over time. In contrast, the omega fatty acids in liquid salmon oil in a mixture with animal feed or with premix are not detected at 4 weeks after mixture with the feed composition, likely due to oxidation of the fatty acids.

| Concentration of Omega Fatty Acids in Feed Composition & Stability over Time |                     |                                     |  |  |  |
|--|---------------------|-------------------------------------|--|--|--|
|  | Prilled Concentrate | Control Feed no Prilled Concentrate | Feed plus Prilled Concentrate <sup>1</sup> | Feed plus Liquid Salmon Oil <sup>2</sup> | Premix plus Liquid Salmon Oil <sup>3</sup> |
| 5  | Wk 0                |                                     |  |  |  |
|  | C20:5               | 2.74                                | <0.01                                      | 0.29                                     | Not sampled                                |
|  | C22:6               | 2.93                                | <0.01                                      | 0.31                                     | Not sampled                                |
| 10   | Wk 4                |                                     |  |  |  |
|  | C20:5               | 2.66                                | <0.01                                      | 0.26                                     | <0.01                                      |
|  | C22:6               | 2.81                                | <0.01                                      | 0.28                                     | <0.01                                      |
|  | Wk8                 |                                     |  |  |  |
|  | C20:5               | 2.75                                | <0.01                                      | 0.26                                     | <0.01                                      |
|  | C22:6               | 2.95                                | <0.01                                      | 0.29                                     | <0.01                                      |

<sup>1</sup> Prilled SO inclusion rate 10.75%.

15 <sup>2</sup> Liquid SO inclusion rate 3.75%.

<sup>3</sup> Liquid SO inclusion rate 2.8%.

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